STRESS RESPONSE OF A COMMON FISH TO CHANGING URBAN STREAM TEMPERATURES

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Table of Contents

Abstract

Urban streams often have higher average and more variable temperatures than forested streams. However, the effects of anthropogenically influenced water temperatures on fish condition are not well understood. This study aimed to quantify the impacts of urbanized stream temperatures on the physiological stress response of Creek Chubs. Trial 1 was conducted for six weeks and the treatment group was subjected to a temperature regime of (17-26°C), while Trial 2 was conducted for 9 weeks and the treatment group was subjected to a temperature regime of (24-26°C). In both trials, the control groups were subjected to a constant 21°C, the optimal growth temperature of Creek Chubs, while the treatment groups' thermal regimes were based on diurnal temperature profiles that simulated urban stream temperatures in Columbus, Ohio. Body condition (length and weight) along with blood-plasma glucose concentrations were measured as indices of stress. Over the duration of the trial, the treatment group Creek Chubs in Trial 1 on average gained 0.68 g more weight and exhibited blood-plasma glucose concentrations 16.4% lower than that of the control group. Meanwhile, over the course of Trial 2, Creek Chubs in the treatment group on average gained 1.66 g less weight and exhibited blood-plasma glucose concentrations 25.1% higher than those in the control group. The results of this study offer insight into some the potential underlying mechanisms regarding thermal stress while demonstrating the need for further research.

Introduction

Although the growth rate of the human population has slowed, over 2 billion additional people are expected to inhabit the Earth by 2050 (Gerland et al. 2014). Additionally, the urban lifestyle is becoming an increasingly predominant mode of living (Nowak & Walton 2005). As a result of the growing population, combined with the increase in urban living, urban land cover in the contiguous United States is expected to increase from 3.1% in 2000 to 8.1% in 2050 (Nowak

& Walton 2005). In Ohio, 77.9% of residents lived in urban areas in 2010, and from 2000-2010 the urban land area of the three most populous cities – Cleveland, Cincinnati, and Columbus – increased by 19.3, 17.3, and 28.4%, respectively (US Census Bureau 2010).

Urbanization is the driving force behind a variety of anthropogenic stressors to streams including increased impervious-surface area, reduction of riparian zone size and quality, and altered channel morphology and hydrology (Urban Stream Syndrome; Walsh et al. 2005). Many of these stressors can contribute to alterations in the temperature regimes of urban streams. For instance, impervious-surface cover can cause unnatural spikes in stream temperature due to runoff that travels over hot asphalt before flowing into streams (Somers 2013). Furthermore, warmer ambient air and ground temperatures coupled with reduced shading contribute to increased average water temperatures and lead to the "urban heat island effect" (Somers, 2013). As a result, streams and rivers (hereafter, "streams") in urban settings typically experience increased variability in temperature as well as higher average water temperatures (Paul & Meyer 2011).

The warming of streams can have significant impacts on ecosystem processes such as stream metabolism and nutrient cycling (Kaushal et al. 2010). In addition, increased temperature variability and higher average temperatures can strongly influence aquatic biota (Krause et al. 2004). Fish, for example, can only tolerate species-specific, finite ranges of temperatures (Neuheimer et al. 2011). Whereas fish kills as a consequence of water temperatures exceeding the upper threshold are rare, sublethal stress due to elevated or more variable water temperatures can have substantial impacts on stream fish from individuals to communities (Beitinger et al. 2000).

Sublethal stress imposed by altered water temperatures can make tolerant species more competitive, creating the potential for shifts in fish assemblages to occur (Taniguchi, 1998). Consequently, urban streams commonly exhibit increased dominance of tolerant species and

reduced biotic richness (Walsh et al. 2005). Additionally, chronic exposure to sublethal temperatures could reduce growth, decrease immune system response, or hinder reproduction (Krause et al. 2004). Physiological responses of fish to sublethal stressors, such as elevated temperatures, are dependent on the severity and duration of the stressor, but can be quantified by measuring a number of stress-response indices such as growth rates or blood-plasma glucose concentrations, both of which have been established as sensitive and reliable indicators of fish stress both in the field and in laboratory conditions (Neuheimer et al. 2011, Cox & Coutant 1981, Feminella & Matthews 1984, Wells & Pankhurst 1999).

In the past few decades, significant developments have been made regarding the ability to predict the extent of thermal impairment as a result of urbanization. Krause et al. (2004) used the Stream Network Temperature Model in conjunction with the Hydrologic Simulation Program Fortran to examine the influences of urban development on thermal habitat in a warm water stream. The researchers concluded that they could accurately predict the mean stream temperature increase as well as increased frequency of maximum daily temperatures greater than 31°C in summer months. Whereas the thermal impacts of urbanization can be modeled with reasonable accuracy, more research on the effects that chronic exposure to elevated temperature regimes has on fish is needed to predict changes in community composition and overall ecosystem integrity.

A holistic understanding of how the environmental impacts of urbanization affect urban streams is imperative to guide future research as well as inform management decisions. A more comprehensive grasp on the effect of temperature on fish in urban streams is integral to that understanding. With that in mind, I aimed to investigate and quantify the impacts of urban stream-temperature variability on the stress responses of a common fish species, Creek Chub (*Semotilus atromaculatus*). Creek Chub represent an ideal study organism due to their thermal tolerance and wide distribution across North America (McMahon 1982, Taniguchi et al. 1998). If

thermal variability that is typical of urban streams elicits a stress response from Creek Chub, it is likely that less tolerant fish species will be negatively affected and to a greater extent. Specifically, I hypothesized that higher average and more variable stream temperatures that are typical of urban streams would elicit a physiological stress response in Creek Chub by exhibiting decreased growth rates and higher blood-plasma glucose levels.

Methods

Creek Chub were captured from Adena Brook, a headwater tributary that drains into the Olentangy River in Columbus, Ohio using a LR-24 Smith-Root (Vancouver, Washington) backpack electrofisher. The treatment group consisted of 30 Creek Chub housed in individual 2-L tanks in an Aquatic Habitat (AHAB) Unit (ZF0601; Apopka, Florida) in the Heffner Building of the Schiermeier Olentangy River Wetland Research Park (ORWPR) at The Ohio State University (OSU). The control group consisted of six Creek Chub housed in identical 2-L tanks that were kept in a system built of polyvinyl chloride (PVC) pipes and food-grade silicon tubing, which was designed to mimic the Aquatic Habitat (AHAB) unit. All tanks were filled with a uniform amount of gravel substrate (~1" deep) and one large rock. Fish from both treatments were placed in a holding tank for two weeks prior to the start of the experiment to allow fish to acclimatize to lab conditions.

Two trials were conducted; the treatment group was subjected to different temperatures in each trial. Both temperature regimes were modeled using continuous temperature data from a headwater tributary of the Olentangy River that flows through a wooded plot of OSU's Waterman Farms during summer months. Temperatures for the treatment group in Trial 1 (17-26 ± 1°C) were chosen to more closely examine the role that temperature variability plays in fish, whereas temperatures for the treatment group in Trial 2 (24-26 ± 1°C) were chosen to investigate the effect of extended periods of warmer temperature. In both trials, temperatures of the

treatment groups fluctuated daily, with temperatures reaching their respective lows from approximately 00:00-06:00 (midnight to 6am), and rising throughout the day to reach their respective peaks around 19:00 (7:00pm). In both trials, the control group was kept constant at 21 ± 1°C, the optimal growth temperature for Creek Chubs (McMahon 1982).

For Trial 1, 30 Creek Chub (108.1 ± 6.2 mm, 10.8 ± 1.8 g; [mean ± SD]) were used in the treatment group. Six Creek Chub (108.2 ± 6.4 mm, 10.6 ± 1.3 g) were used in the control group. Two fish, one in the treatment group and one in the control group, did not complete the trial due to mortality and were not included in the subsequent statistical analysis. For Trial 2, 30 Creek Chub (118.3 ± 7.5 mm, 16.8 ± 3.1g) were used in the treatment group. Six Creek Chub (120.8 ± 6.9 mm, 17.3 ± 2.4 g) were used in the control group. Seven fish, all in the treatment group, did not complete the trial due to mortality and were not included in the statistical analysis.

Length and weight were measured weekly and blood-glucose was measured on days 7, 21, and 42 for Trial 1 and days Pre-Trial (PT), 0, 21, 42, and 63 for Trial 2. Individual Creek Chub were removed from their respective tanks and placed in a 1-L beaker containing a 100-mg L^{-1} solution of MS-222. Fish were immersed in solution until a surgical plane of anesthesia was reached and fish lost balance (approximately 2 min). Fish were removed from the anesthetizing agent, and a 25-gauge needle was inserted ventrally into the caudal peduncle posterior to the anal fin in order to draw blood from the caudal vein. A microcuvette was filled using the drawn blood and read in a HemoCue Glucose 201 meter (Ängelholm, Sweden). Fish were then placed in a recovery beaker of tank water before being placed back into their study tank.

Water-quality parameters were monitored throughout the experiment to ensure homogenous conditions between treatments and between Trials 1 and 2. In order to verify optimal water conditions, YSI 5200A Multiparameter Monitors (Yellow Spring, Ohio) were used to record temperature, pH, dissolved oxygen (mg L^{-1}), and conductivity (µs cm^{-1}) on a daily basis. Air pumps maintained dissolved oxygen concentrations at ≥6.2 (mg L^{-1}) at all times in

both the treatment and control group during trials 1 and 2. Water samples were analyzed for nitrates (NO_3^-), nitrite (NO_2^-), ammonia (NH_3), and chloride (Cl^-) on a weekly basis using HACH Test 'N Tube NitraVer X Reagent Set 2605345, HACH Test 'N Tube NitraVer 3 Reagent Set 2608345, HACH AmVer Low Range Ammonia Test 'N Tube Reagent Set, and HACH Chloride Test Kit Model 8-P 1440-01. All measured components of chemical water quality were within healthy ranges for both the treatment and control groups in Trial 1 and Trial 2 (Appendix: Table A; Appendix: Table B.). All fish were fed 1g of floating shrimp pellets daily. Lab lighting utilized a diurnal pattern consistent with current local natural conditions (14 h light, 10 h dark). All experimental procedures were conducted under IACUC 2010A00000172-R2 and Ohio Division of Wildlife Collection Permit 21-134.

Generalized linear mixed models (GLMM) were used to examine the effects of center length, treatment, time, and individual fish on weight and blood-plasma glucose concentrations. Center length (length-$\bar{X}$length) was used as a more realistic description of change in length rather than describing fish length by its difference from zero, as zero is a value that is not applicable in terms of fish length. For the GLMM with weight as the response variable, center length, treatment, and their interaction (center length × treatment) were included as fixed effects with individual fish and time as random effects. For the GLMM in which glucose was the response variable, treatment was included as a fixed effect with individual fish and time as random effects. All statistical analyses were completed in R statistical software v.3.5.1 (R Core Team, 2018.) using packages *lme4* (Bates et al. 2015) and *MuMIn* (Barton 2018).

Results

Trial 1

Over the course of 42 d, Creek Chub in the treatment group increased in length and weight from 108.1 ± 6.2 mm and 10.8 ± 1.8 g; [mean ± SD] to 110.6 ± 5.8 mm and 13.1 ± 2.4 g,

respectively (Figure 1a,b). Creek Chub in the control group increased in length and weight from 108.2 ± 6.4 mm and 10.6 ± 1.3 g to 111.0 ± 7.5 mm and 12.3 ± 2.5 g, respectively (Figure 1a,b). The average absolute growth rates for Creek Chub in the treatment and control groups throughout the duration of the study were 0.38 (g week^{-1}) and 0.23 (g week^{-1}), respectively. Both center length (GLMM: p = <0.001) and treatment (GLMM: p = 0.047) had a significant effect on weight (Table 1). Center length, treatment, and center length × treatment accounted for 78% of the variation in weight exhibited across both trials, while variation among random effects, individual fish and time, accounted for an additional 13% of variation (Table 2).

Treatment group blood-plasma glucose levels decreased from 55.7 ± 16.9 mg dL^{-1} to 47.6 ± 9.3 mg dL^{-1} and control group blood-plasma glucose levels increased from 63.3 ± 5.0 mg dL^{-1} to 66.8 ± 8.2 mg dL^{-1} (Figure 2). Treatment (GLMM: p = 0.019) had a significant effect on glucose (Table 1). The fixed effect, treatment, explained only 8% of variation, with random effects, individual fish and time, accounting for an additional 16% of variation (Table 2). No individual measurements of weight or glucose in either the treatment or control groups deviated substantially from the response of the group (Appendix: Figure A, Appendix Figure B).

Trial 2

Over the course of 63 d, length and weight of Creek Chub in the treatment group increased from 118.3 ± 7.5 mm and 16.8 ± 3.1g; [mean ± SD] to 120.8 ± 7.6 mm and 16.9 ± 3.5 g (Figure 3a,b), respectively. In comparison, length and weight of Creek Chub in the control group increased from 120.8 ± 6.9 mm and 17.3 ± 2.5 g to 127.3 ± 6.3 mm and 19.0 ± 3.9 g (Figure 3a,b), respectively. The average absolute growth rate for Creek Chub in the treatment and control groups throughout the duration of study were 0.01 (g week^{-1}) and 0.19 (g week^{-1}), respectively. Center length, treatment, and center length × treatment accounted for 53% of the

variation in weight exhibited across both trials, while variation among individual fish and time (random effects) accounted for an additional 36% of variation (Table 2).

Treatment group mean blood-plasma glucose levels increased from 42.4 ± 16.7 mg dL^{-1} to 51.8 ± 9.7 mg dL^{-1} and control group mean blood-plasma glucose levels increased from 37.0 ± 14.1 mg dL^{-1} to 39.8 ± 10.4 mg dL^{-1} (Figure 6). Treatment had a significant effect ($p = 0.034$) on glucose concentrations (Table 1). The fixed effect, treatment, explained only 8% of variation, with random effects, individual fish and time, accounting for an additional 38% of variation (Table 2). No individual measurements of weight or glucose in either the treatment or control groups deviated substantially from the response of the group (Appendix: Figure C, Appendix: Figure D).

Discussion

Exposure to stress can prompt a spate of physiological responses in fish. Typically when fish endure chronic stress, whether by heat stress or any other stressor, their growth, and consequentially weight, can be negatively affected by symptoms such as reduced appetite and consumption, diminished assimilation efficiency, and increased metabolic rate (Wendelaar Bonga 1997). Another form in which stress can manifest itself physiologically is elevated glucose concentrations (Wendelaar Bonga 1997). For fish, when a stressful situation is encountered, the body employs a number of mechanisms to meet the increased energy demand of stressful situations (Iwama 1998). One of those mechanisms is for the liver to increase glucose production in order to supply critical tissues, such as the brain, muscles, and gills, with energy subsidies in times of stress (Iwama 1998). In summary, when fish experience decreased weight gain and elevated blood-plasma glucose concentrations, these indices serve as reliable indicators of a physiological stress response (Wendelaar Bonga 1997). While fish in both treatment groups

were originally anticipated to exhibit reduced weight gain and elevated blood-plasma glucose concentrations compared to control groups, the treatment group in Trial 1 exhibited increased weight gain (GLMM: $p = 0.047$) and lower blood-plasma glucose concentrations (GLMM: $p = 0.019$) than the control group. Trial 2 found no differences in weight gain between treatments (although length was a significant effect) but the treatment group had significantly higher blood-plasma glucose concentrations (GLMM: $p = 0.034$).

Theoretically, the Trial 1 control group should have exhibited the optimal growth rate for Creek Chubs, owing to the fact that control groups were subjected to a constant 21 °C, the ideal temperature for maximum growth in Creek Chub, and the treatment groups thermal regime (17-26°C) extends well beyond that. In contrast, I observed that Creek Chub in the control group gained significantly more weight than the control group. There are a variety of reasons that could potentially explain why the treatment group in Trial 1 did not elicit a physiological stress response. For example, several studies have found that fish exposed to fluctuating temperatures experience increased growth rates compared to fish subjected to constant temperatures of the same mean as the fluctuating temperatures (Cox & Coutant 1981, Hokansen et al. 1977), as thermocycles can allow for more rapid consumption of food and higher energetic conversion efficiencies resulting in increased weight gain and lipid deposition (Spigarelli et al. 1982). This process may explain why the treatment group in Trial 1 gained more weight than the control group (Diana 1984, Spigarelli et al. 1982).

In addition to temperature variability – or lack thereof – the maximum temperature of the treatment group of Tria1 1 may simply not have been high enough to elicit a sublethal stress response in the Creek Chubs. Creek Chubs were subjected to a maximum temperature of 26°C, and while certainly higher than their optimal growth temperature of 21°C, the maximum temperature was still far below the Creek Chub critical thermal maxima of 35.6°C (Beitinger et al. 2000). As the boundary for the point between the optimal growth temperature and the critical

thermal maxima in which sublethal effects arise is not only ill defined but also pliable, it is entirely possible 26°C is below the physiological threshold at which sublethal stress commences in Creek Chubs. In partial support of this explanation, we also observed that Creek Chub from Trial 2, which were exposed more consistently to higher temperatures (24-26°C) but still to the same maximum temperature as Trial 1 (17-26°C), did not exhibit reduced growth rates when compared to the control either.

The difference in blood-plasma glucose concentrations between the control and treatment groups in Trial 2 may be due to a physiological stress response elicited by Creek Chub due to thermal stress. However, while the treatment group in Trial 2 experienced a 22.2% increase in blood-plasma glucose concentrations, it is important to note the ecological significance of the blood-plasma glucose concentration increasing from 42.3 to 51.8 mg dL^{-1} remains unclear, as an increase of such magnitude may not translate to deleterious health effects if the elevated blood-plasma glucose concentrations still fall within a range that is not unnatural for Creek Chub. Published literature concerning naturally occurring Creek Chub blood-plasma glucose concentrations is lacking, and whereas information on blood-plasma glucose concentrations in fish of the family Cyprinidae is available, Pottinger (2010) demonstrated blood-plasma glucose concentrations can vary significantly even between species of the same family. Another possible mechanism explaining this pattern may be physiological variation among individual fish as blood-plasma glucose concentrations can also vary significantly within the same species and even between the sexes (Chavin & Young 1970, Afonso et al. 2003). Additionally this explanation is supported because the control group in Trial 1 and Trial 2 were subjected to identical conditions yet the mean blood-plasma glucose levels of the control group in week 6 of Trial 1 was 66.8 ± 8.2 mg dL^{-1} while the mean blood-plasma glucose levels of the control group at the same point in Trial 2 was significantly lower at 39.8 ± 10.4 mg dL^{-1}. Furthermore, the

effects of individual variation had the potential to be amplified by the small sample size ($n = 6$) of the control groups.

Owing to the fact that biochemical reactions transpire as a function of body temperature, even temperature shifts of just a few degrees can have numerous detrimental effects on fish including reduced growth rates, decreased immune system response, diminished fecundity, decreased egg quality and reduced sperm motility (Krause et al. 2004, Ficke et al. 2007, Schreck 2010). Furthermore, prolonged stress on the population scale and the decreased fitness that occurs as a result can cause marked shifts in abundance, occurrence, size, distribution and survival (Lyons et al. 2010).

However, there are several mechanisms by which fish are capable of mitigating heat stress if they are exposed to temperatures above their optimal range. For example, fish are capable of acclimating to temperatures that are outside their thermal range up until a point when the limit of their physiological plasticity is reached (Beitinger et al. 2000). Beyond this point and before the critical thermal maximum is reached, thermal stress is incurred (Beitinger et al. 2000). However, the lower limit of thermal stress and the upper critical thermal maximum can vary considerably, even within the same species, due to individual variation and past thermal history (Feminella & Matthews 1984) (Beitinger et al. 2000). Several field studies across multiple species of fish have demonstrated that two fish populations of the same species in the same watershed can have significantly different critical thermal maxima due to extremely localized acclimation (Feminella & Matthews 1984, Strange et al. 2002, Eliason et al. 2011). Fish size can play a significant role in critical thermal maximum within species as well (Underwood et al. 2012). Further complicating matters, if fish experience thermal stress and then are granted reprieve in the form of lower temperatures within the range of the fish's physiological limits, this may serve as a recovery period in which the effect of sublethal stress are alleviated (Bevelhimer & Bennett 2000). Although the specifics concerning how low the temperature needs to be for full

recovery to occur, the rate of recovery and the period of time necessary to fully recover from sublethal effects are poorly understood (Bevelhimer & Bennett 2000).

In conclusion, Creek Chub in the Trial 1 treatment group gained more weight and had lower blood-plasma glucose concentrations than the control group, while Creek Chub in the Trial 2 treatment group showed no significant difference in weight compared to the control treatment, but the treatment group had significantly higher blood-plasma glucose concentrations. A stress response in a tolerant species like Creek Chub could potentially translate to dire impacts for more sensitive species as even minor increases in chronic stress caused by heat or other factors can have substantial consequences for wild fish. Furthermore, behavioral adaptations that allow fish to move from stream habitats with elevated temperatures to cooler areas (e.g., deeper, cooler pools) will likely be increasingly limited as the effects of urbanization and climate change increase in intensity and coverage (Ficke et al. 2007). Thus, although individual variation and physiological plasticity, among other mechanisms that dictate sublethal stress responses, make it inherently difficult to fully decipher the impacts of heat stress, developing a stronger understanding of the consequences of heat stress on stream fishes is critical for their protection, and this is likely especially the case for more sensitive species. It will also be critical to investigate the effects of the interaction of altered thermal regimes and the suite of other stressors to which urban streams are subjected. Although the results of Creek Chub stress response to urban thermal regimes are important independently, it is paramount to consider the potential implications of thermal stress in the context of potential interactions with the multiple stressors that are typical of urban environments, such as altered channel morphology and hydrology, elevated influx of nutrients and contaminants, and increased siltation, on stream fishes and ecosystems.

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Tables

Table 1. Analysis of variance results (degrees of freedom calculated from Satterthwaite's method) from generalized linear mixed models (GLMMs). Models tested for the effects of center length, treatment, and center length × treatment on fish weight and the effects of treatment on blood-plasma glucose concentrations. Individual fish and time were treated as random effects in both models. SS = Sum of Squares, MS = Mean Squares, Num *d.f.* = degrees of freedom in the numerator, Den *d.f.* =degrees of freedom in the denominator. Bold-faced p-values indicate significant results at $\alpha = 0.05$.

Response Variable and Fixed Effect	**SS**	**MS**	**Num *d.f.***	**Den *d.f.***	***F***	***P***	**Random Effect**	**Variance**	**S.D.**
Trial 1									
Weight									
Center Length	60.32	60.32	1	34.60	144.43	**<0.001**	Individual Fish	0.857	0.926
Treatment	1.80	1.80	1	28.27	4.32	**0.047**	Time	0.716	0.268
Center Length*Treatment	0.89	0.89	1	34.60	2.13	0.154	Residual	0.418	0.646
Glucose									
							Individual Fish	134.040	11.58
Treatment	817.76	817.76	1	39.93	6.0	**0.019**	Time	4.495	2.12
							Residual	136.359	11.68
Trial 2									
Weight									
Center Length	71.86	71.86	1	72.91	70.99	**<0.001**	Individual Fish	2.477	1.574
Treatment	0.159	0.159	1	24.52	0.16	0.695	Time	0.031	0.178
Center Length*Treatment	0.248	0.248	1	72.91	0.25	0.622	Residual	1.012	1.006
Glucose									
							Individual Fish	99.106	9.955
Treatment	513.65	513.65	1	19.36	5.22	**0.034**	Time	1.396	1.181
							Residual	98.481	9.924

Table 2. Marginal (fixed effects only) and conditional (fixed and marginal effects) R^2 values for weight and glucose generalized linear mixed-effects models for Trial 1 and Trial 2.

Models	R^2 **- Marginal**	R^2 **- Conditional**
Trial 1		
Weight	0.78	0.91
Glucose	0.08	0.24
Trial 2		
Weight	0.53	0.89
Glucose	0.08	0.46

Figures

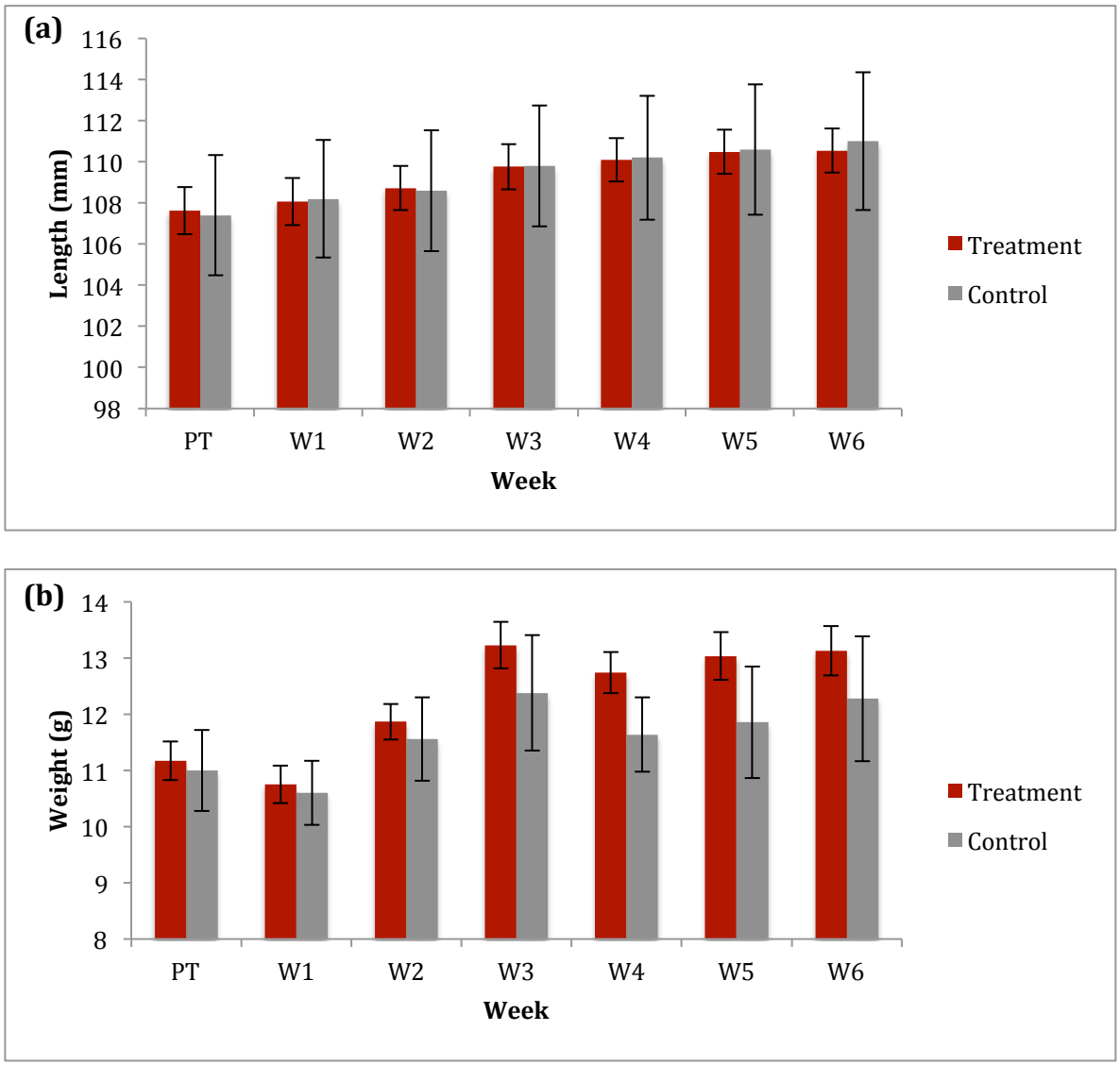

Figure 1. Trial 1 Creek Chub (a) mean length by week and (b) mean weight by week in the treatment (red) and control (gray) tanks (+/- 1 SE). PT = Pretreatment. W = Week.

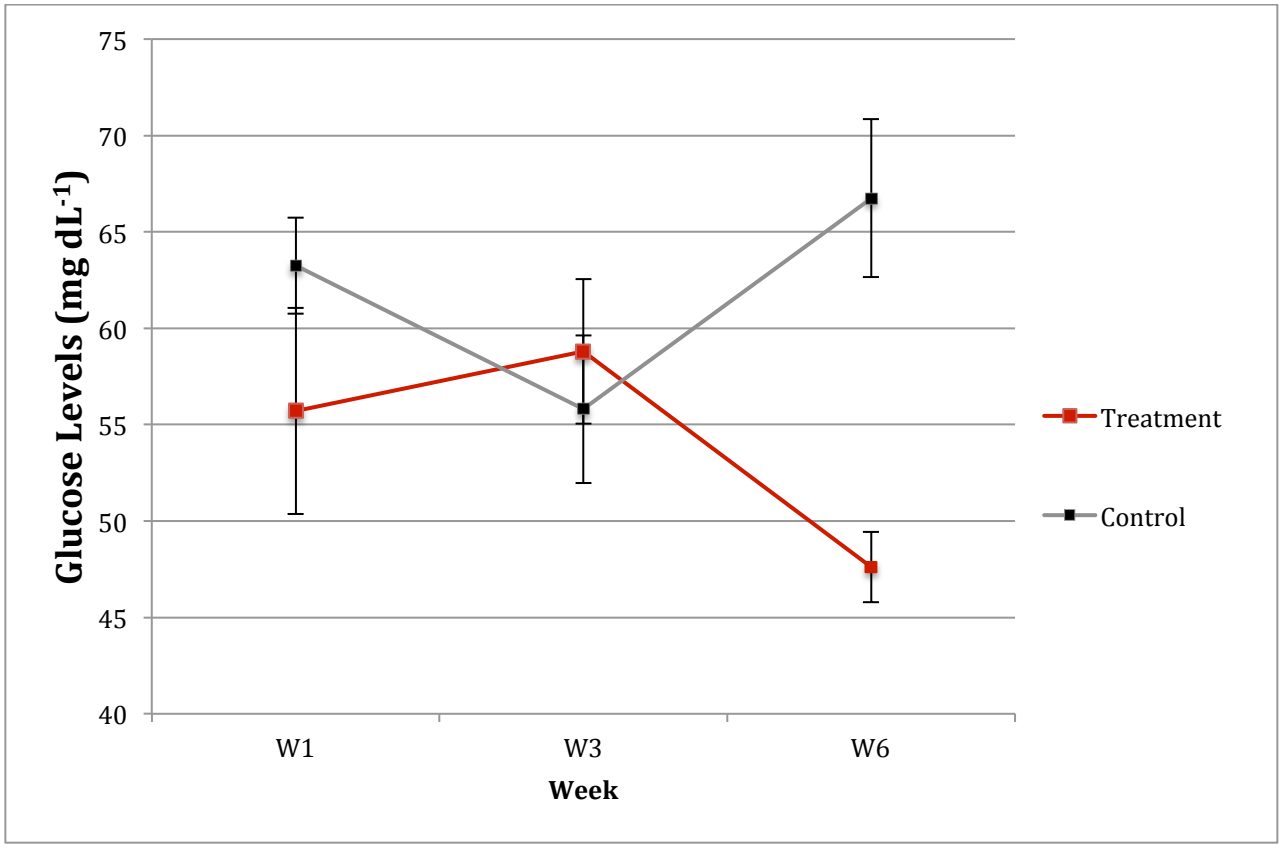

Figure 2. Trial 1 Creek Chub mean blood-plasma glucose levels by week in the treatment (red) and control (gray) tanks (+/- 1 SE). W = Week.

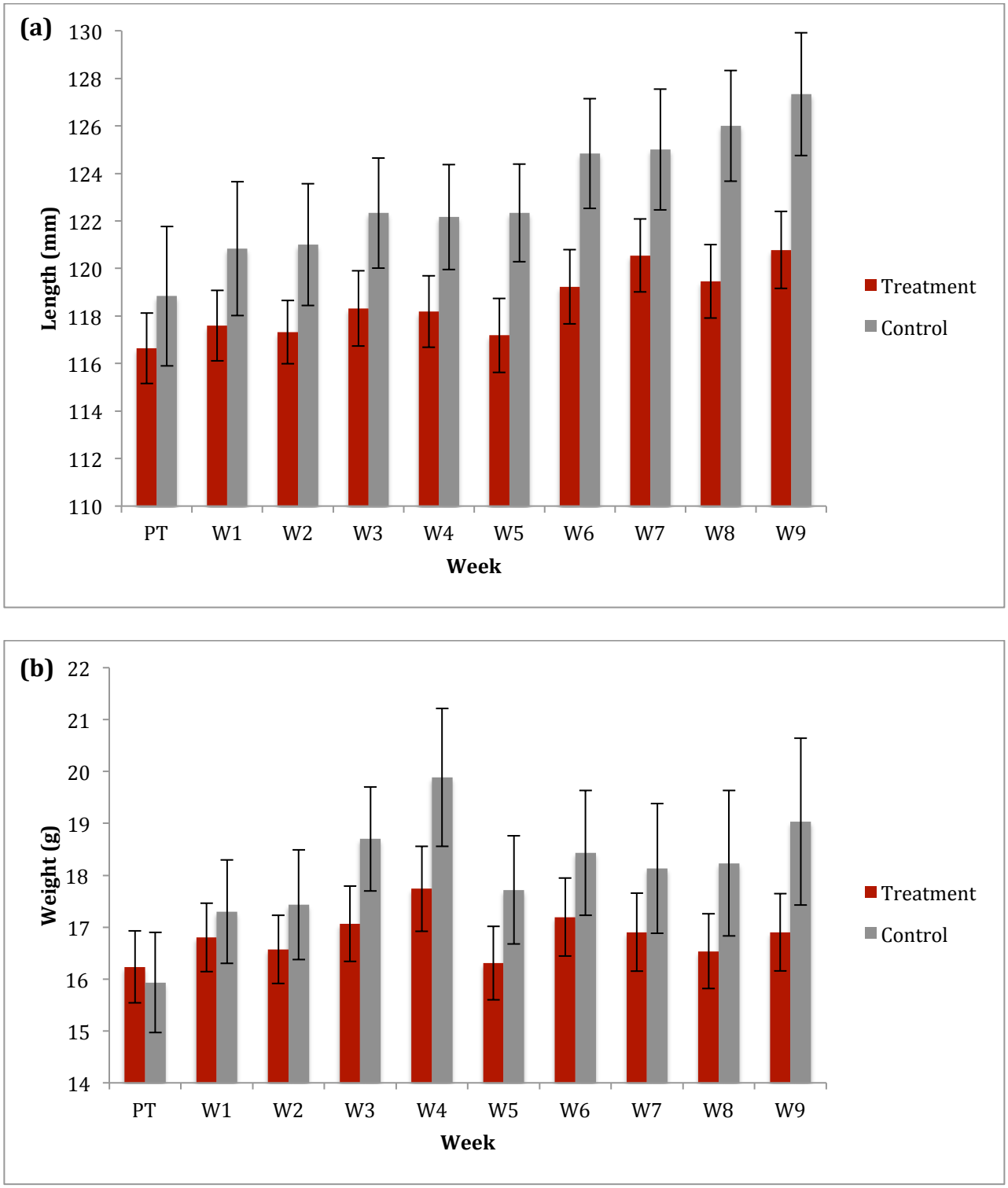

Figure 3. Trial 2 Creek Chub (a) mean length by week and (b) mean weight by week in the treatment (red) and control (gray) tanks (+/- 1 SE). PT = Pretreatment. W = Week.

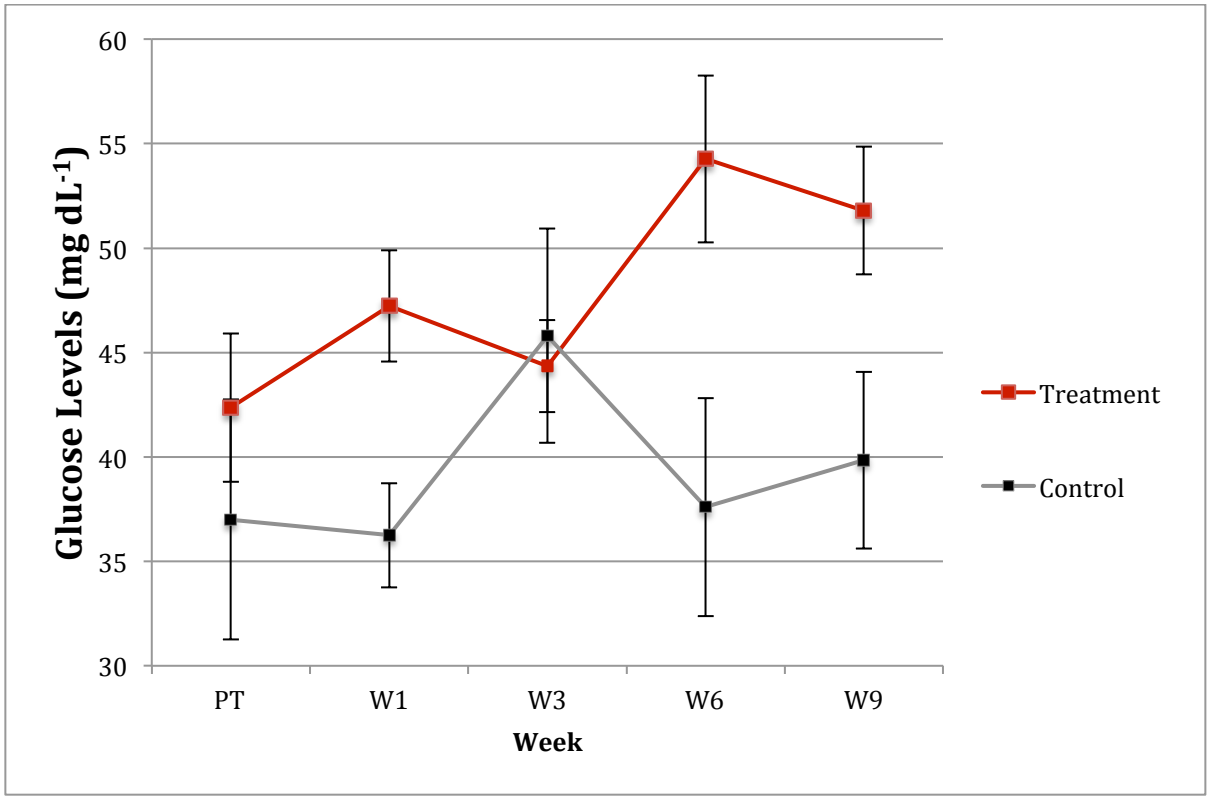

Figure 4. Trial 2 Creek Chub mean blood-plasma glucose levels by week in the treatment (red) and control (gray) tanks (+/- 1 SE). PT=Pretreatment. W = Week.

Appendix

Table A. Weekly measurements of water quality parameters for Trial 1.

	Ammonia (ppm)	**Nitrates ($mg\ L^{-1}$)**	**Nitrites ($mg\ L^{-1}$)**	**Chloride ($mg\ L^{-1}$)**	**Salinity (ppt)**
Pretreatment	0.0	10.0	0.0	25.0	0.1
Week 1	0.0	10.0	0.0	20.0	0.1
Week 2	0.0	10.0	0.0	20.0	0.1
Week 3	0.0	10.0	0.0	20.0	0.1
Week 4	0.0	20.0	0.0	25.0	0.1
Week 5	0.0	10.0	0.0	20.0	0.1
Week 6	0.0	20.0	0.0	20.0	0.1

Table B. Weekly measurements of water quality parameters for Trial 2.

	Ammonia (ppm)	**Nitrates ($mg\ L^{-1}$)**	**Nitrites ($mg\ L^{-1}$)**	**Chloride ($mg\ L^{-1}$)**	**Salinity (ppt)**
Pretreatment	0.0	6.6	0.0	15.0	0.1
Week 1	0.0	6.3	0.0	20.0	0.1
Week 2	0.0	5.6	0.0	15.0	0.1
Week 3	0.0	5.6	0.0	15.0	0.1
Week 4	0.0	4.7	0.0	20.0	0.1
Week 5	0.0	6.9	0.0	15.0	0.1
Week 6	0.0	6.6	0.0	15.0	0.1
Week 7	0.0	6.4	0.0	20.0	0.1
Week 8	0.0	5.1	0.0	10.0	0.1
Week 9	0.0	5.3	0.0	15.0	0.1

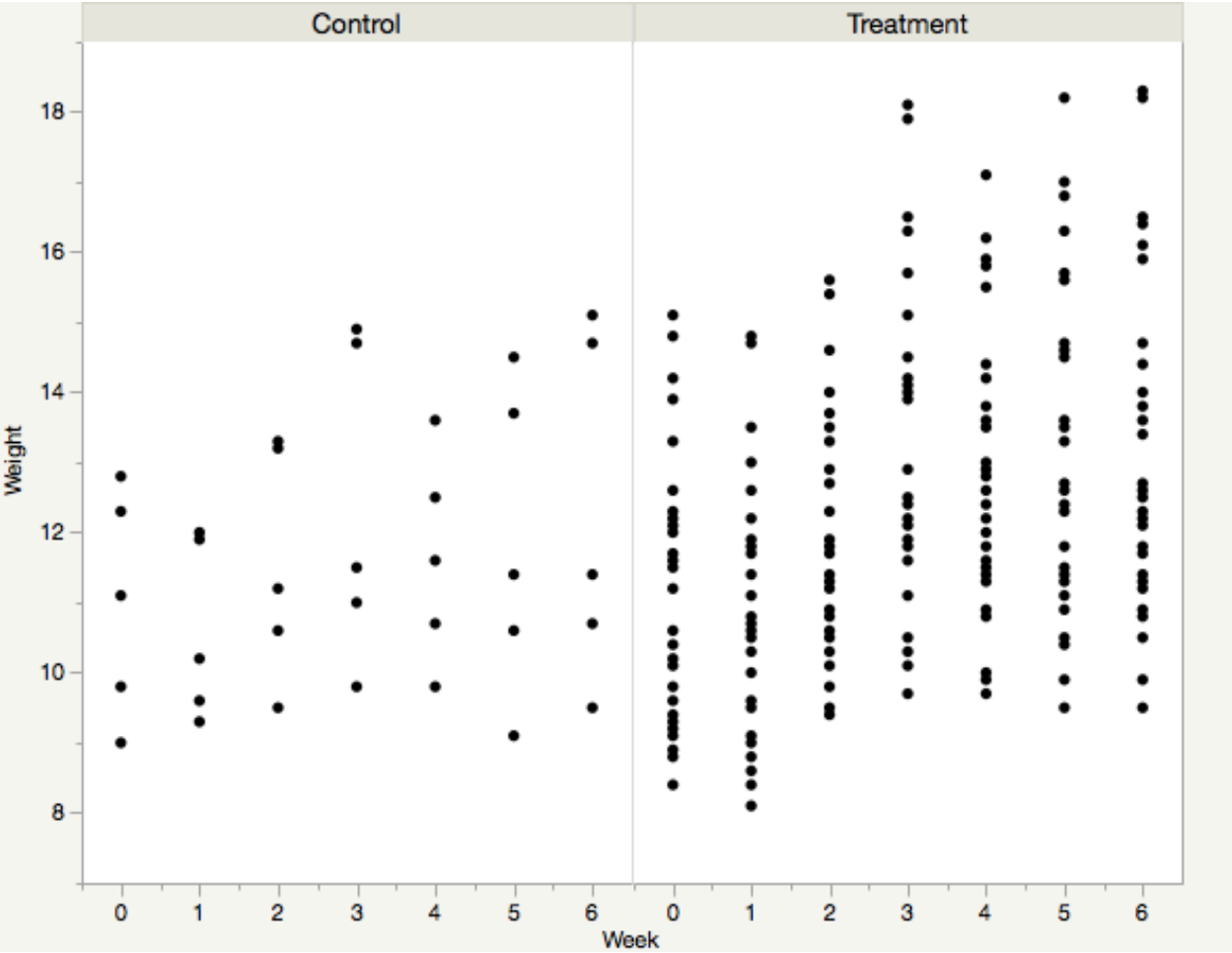

Figure A. Trial 1 individual weights (g) of fish in control and treatment groups by week.

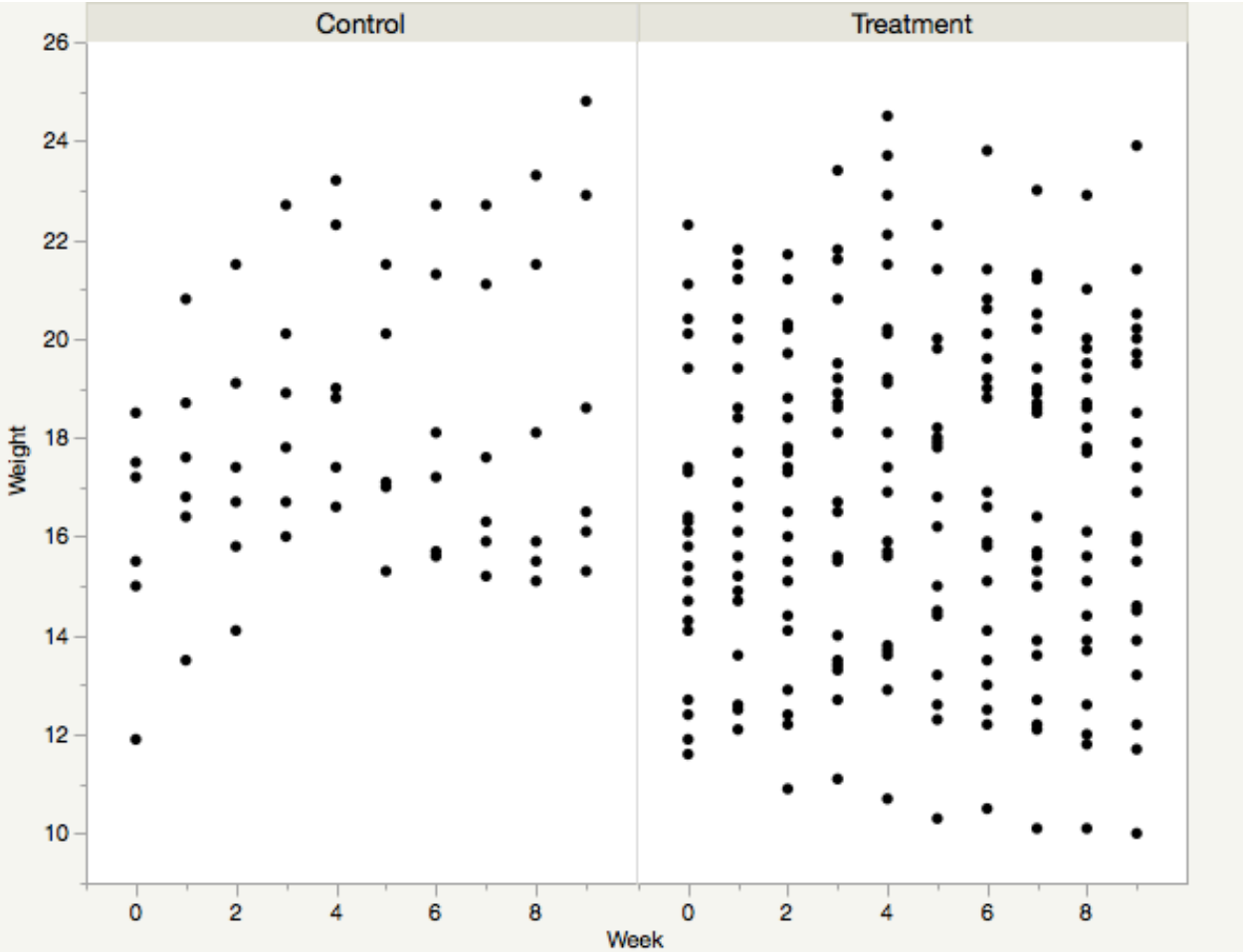

Figure B. Trial 2 individual weights (g) of fish in control and treatment groups by week.

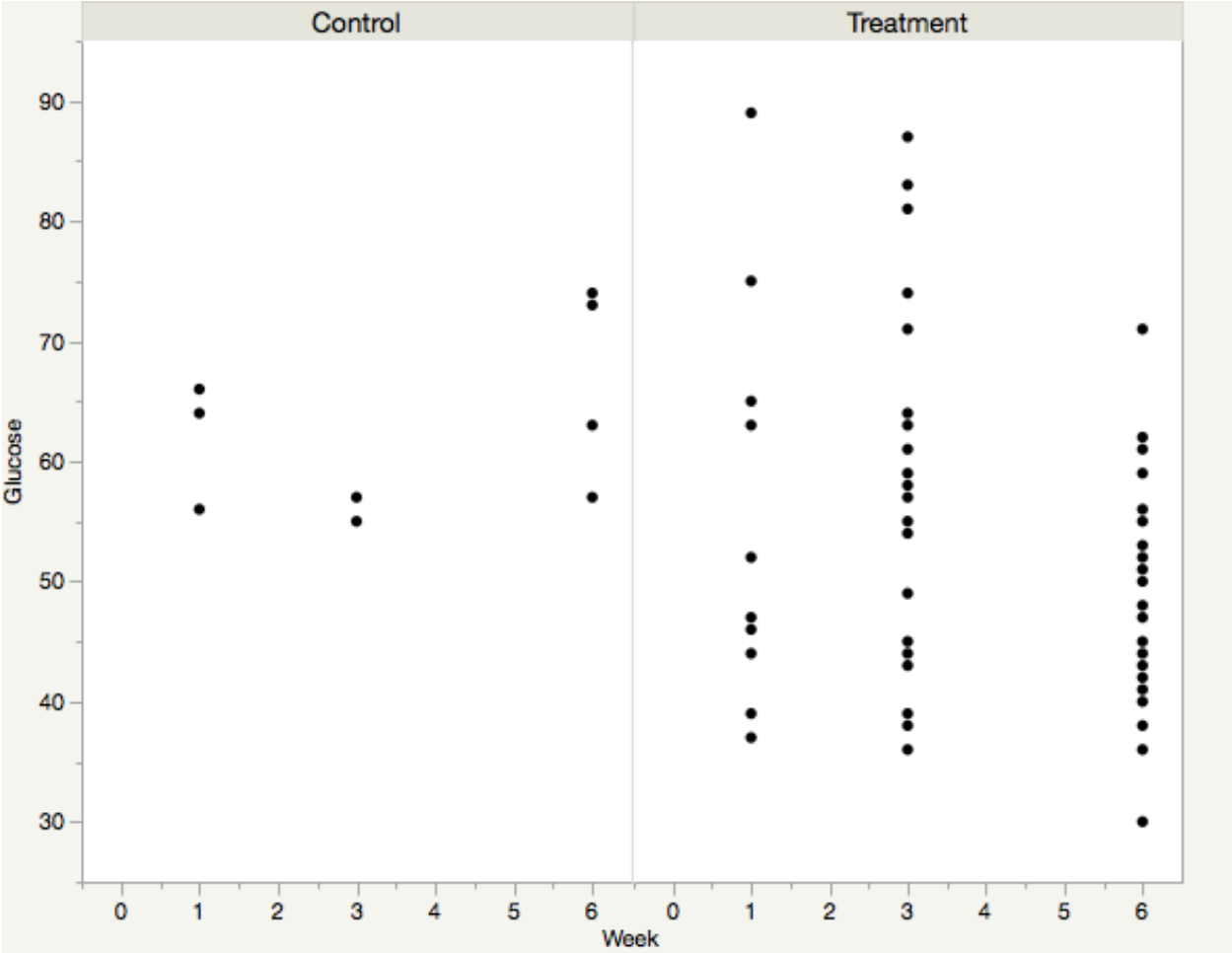

Figure C. Trial 2 individual blood-plasma glucose concentrations (mg dL^{-1}) of fish in control and treatment groups by week.

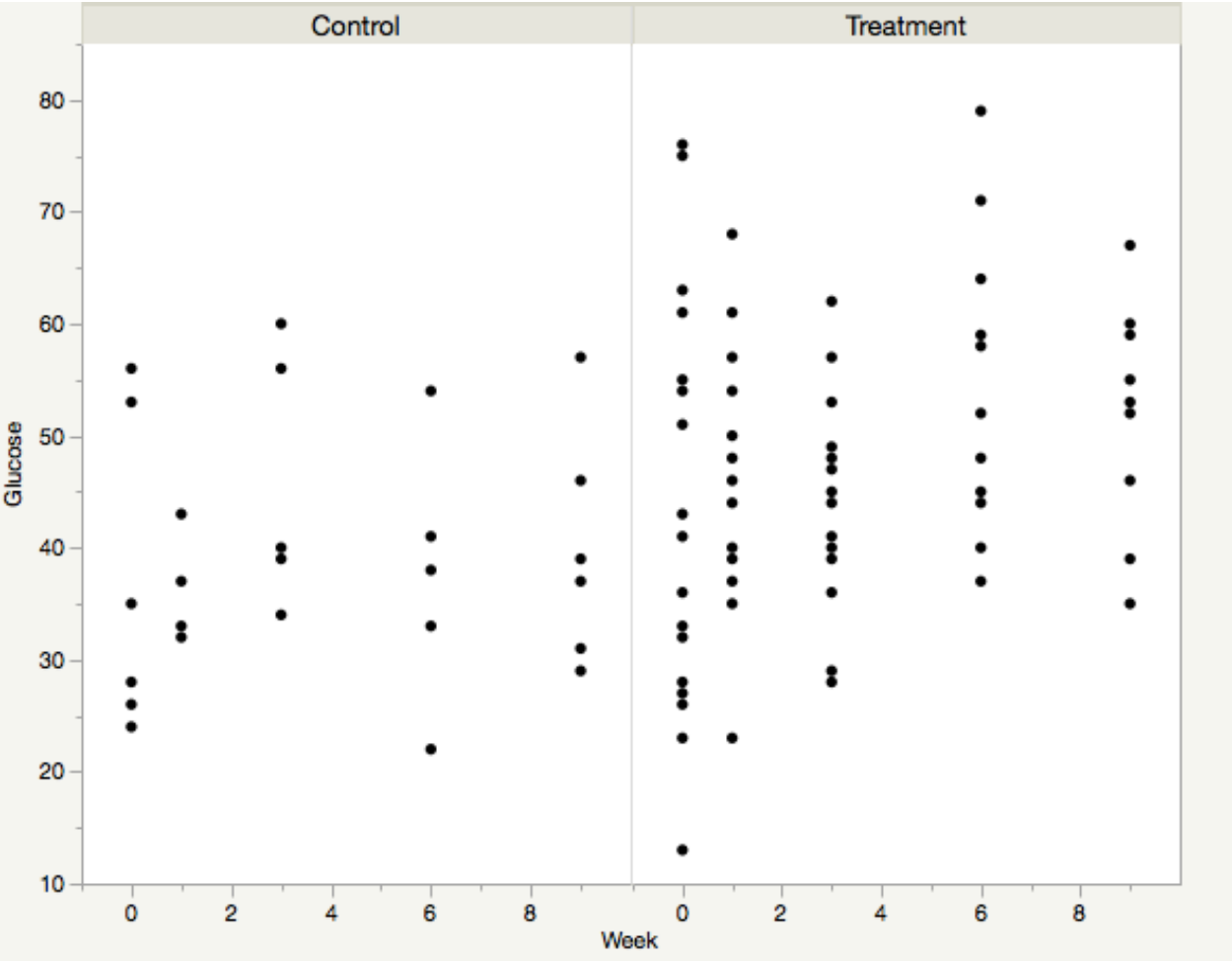

Figure D. Trial 2 individual blood-plasma glucose concentration (mg dL^{-1}) of fish in control and treatment groups by week.